CLAIMS

What is claimed is:

1. A vector for reverting cell lines to a pro-B cell-like state or to a germline-like state, said vector comprising one or more immunoglobulin regions selected from the group consisting of V regions, D regions, and J regions, a 5' flanking region operably linked 5' to a 5'-most region and a 3' flanking region operably linked 3' to a 3'-most region wherein said 5' and 3' flanking regions are capable of facilitating homologous recombination of said immunoglobulin regions into a cell having a V(D)J rearranged immunoglobulin gene.

2. The vector of claim 1 wherein said vector comprises one or more fused DJ regions.

3. The vector of claim 1 wherein said vector comprises two, three, four, five or more fused DJ regions.

4. The vector of claim 1 wherein said vector comprises six fused DJ regions.

5. The vector of claim 1 further comprising a 5' flanking region and a 3' flanking region.

6. The vector of claim 1 further comprising a selectable marker operably linked 3' to said 5' flanking region and 5' to said 5'-most region.

7. The vector of claim 6 wherein said selectable marker is operably linked 5' to the 5'-most DJH region.

8. The vector of claim 6 wherein said selectable marker is *Eco-gpt*.

9. A vector comprising a polynucleotide sequence encoding a recombination-promoting protein, or functional fragment, derivative, or variant thereof, selected from the group consisting of RAG-1 and RAG-2.

10. The vector of claim 9 wherein said vector is a plasmid vector.

11. The vector of claim 9 wherein said vector is a viral vector.

12. The vector of claim 11 wherein said viral vector is an adenoviral vector.

13. The vector of claim 9 selected from the group consisting of pCDNA3-RAG1 (SEQ ID NO: 40), pBI-TdT-GFP.RAG2 (SEQ ID NO: 42), pShuttle-RAG2 (SEQ ID NO: 52), pShuttle-GFP-RAG2 (SEQ ID NO: 53), pAdEasy-RAG2 (SEQ ID NO: 54), pAdEasy.1-GFP-RAG2 (SEQ ID NO: 55), and pAdEasy.2-GFP-RAG2 (SEQ ID NO: 56).

14. A method for generating immunoglobulin heavy and/or light chains, said method comprising the steps of:

(a) reverting a cell comprising a V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding V, D, and/or J regions of an immunoglobulin heavy and/or light chain, or fragment thereof, wherein said V, D, and/or J regions replace said V(D)J rearranged immunoglobulin region such that the introduced V, D, and/or J regions are in a pro-B cell-like or a germline-like state; and

(b) expressing in said reverted cell a polynucleotide sequence encoding a recombination-facilitating protein, or functional fragment, derivative or variant thereof, for a time and under conditions sufficient to induce rearrangement of the V, D, and/or J regions, wherein rearrangement of the V, D, and/or J regions facilitates expression of an immunoglobulin heavy and/or light chain.

15. A method for generating immunoglobulin heavy chains, said method comprising the steps of:

(a) reverting a cell comprising a V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding fused DJ regions of an immunoglobulin heavy chain, wherein said DJ regions replace said V(D)J rearranged immunoglobulin region such that the introduced fused DJ regions are in a pro-B cell-like state; and

(b) expressing in the reverted cell a polynucleotide sequence encoding a recombination-facilitating protein, or functional fragment thereof, for a time and under conditions sufficient to induce rearrangement of the germline V regions in the reverted cell with the introduced fused DJ regions,

wherein rearrangement of the V and fused DJ regions facilitates expression of an immunoglobulin heavy chain.

16. A method for generating immunoglobulin light chains, said method comprising the steps of:

(a) reverting a cell comprising a V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding J regions of an immunoglobulin light chain, wherein said J regions replace said V(D)J rearranged immunoglobulin region such that the introduced J regions are in a pro-B cell-like or a germline-like state; and

(b) expressing in the reverted cell a polynucleotide sequence encoding a recombination-facilitating protein, or functional fragment thereof, for a time and under conditions sufficient to induce rearrangement of the germline V regions in the reverted cell with the introduced J regions, wherein rearrangement of the V and J regions facilitates expression of an immunoglobulin light chain.

17. A method for generating libraries of cells that produce an array of immunoglobulins wherein each immunoglobulin exhibits a particular antigen specificity, said method comprising the steps of:

(a) providing a cell having a V(D)J rearranged immunoglobulin heavy and/or light chain region;

(b) introducing into the cell a polynucleotide encoding V, D, and/or J regions of an immunoglobulin heavy and/or light chain, or fragment thereof, wherein said V, D, and/or J

regions replace said V(D)J rearranged immunoglobulin heavy and/or light chain region such that the introduced V, D, and/or J regions are in a pro-B cell-like or a germline-like state;

(c) culturing the cell under suitable conditions to generate a reverted cell population each member of which population comprises V, D, and/or J regions in a pro-B cell-like or a germline-like state;

(d) introducing into cells of the reverted cell population a polynucleotide sequence encoding a recombination-facilitating protein, or functional fragment, derivative or variant thereof; and

(e) culturing the resulting population of cells expressing the recombination-facilitating protein for a time and under conditions sufficient to induce rearrangement of the pro-B cell-like or germline-like V, D, and/or J regions, wherein rearrangement of the V, D, and/or J regions facilitates expression of an immunoglobulin heavy and/or light chain having a particular antigen specificity.

18. A method for identifying in a cell an immunoglobulin having a desired antigen specificity, said method comprising the steps of:

(a) reverting a cell comprising a V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding V, D, and/or J regions of an immunoglobulin heavy and/or light chain, or fragment thereof, wherein said V, D, and/or J regions replace said V(D)J rearranged immunoglobulin region such that the introduced V, D, and/or J regions are in a pro-B cell-like or a germline-like state;

(b) expressing in the reverted cell a polynucleotide sequence encoding a recombination-facilitating protein, or functional fragment, derivative, or variant thereof, for a time and under conditions sufficient to induce rearrangement of the pro-B cell-like or germline-like V, D, and/or J regions, wherein rearrangement of the V, D, and/or J regions facilitates expression of an immunoglobulin heavy and/or light chain; and

(c) screening the resulting V, D, and/or J region rearranged cells for an immunoglobulin having the desired antigen specificity.

19. A method for generating cell lines capable of producing immunoglobulins having a desired specificity, said method comprising the step of reverting a cell comprising a

V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding V, D, and/or J regions of an immunoglobulin heavy and/or light chain gene, or fragment thereof, wherein said V, D, and/or J regions replace said V(D)J rearranged immunoglobulin region such that the introduced V, D, and/or J regions are in a pro-B cell-like or a germline-like state.

20. A method for generating cell lines capable of producing immunoglobulins having a desired specificity, said method comprising the step of reverting a cell comprising a V(D)J rearranged immunoglobulin region by introducing into said cell a polynucleotide encoding one or more fused DJ regions of an immunoglobulin heavy chain, wherein said fused DJ regions replace said V(D)J rearranged immunoglobulin region such that the introduced fused DJ regions are in a pro-B cell-like state.

21. The method of claim 20 wherein said introduced polynucleotide comprises two or more fused DJ regions.

22. The method of claim 20 wherein said introduced polynucleotide comprises at least three, four, or five fused DJ regions.

23. The method of claim 20 wherein said introduced polynucleotide comprises six fused DJ regions.

24. A method for producing immunoglobulins having a particular affinity or specificity for a target molecule, said method comprising the steps of:

(a) generating a reverted lymphoma cell line capable of producing immunoglobulins;

(b) expressing a polynucleotide encoding a recombination-facilitating protein, or functional fragment, derivative, or variant thereof, in the reverted lymphoma cell line for a time and under conditions sufficient to induce a rearrangement of the genes encoding the immunoglobulins which facilitates the generation of a library of lymphoma cells which produce an array of immunoglobulins wherein each immunoglobulin exhibits a particular affinity or specificity; and

(b) screening for immunoglobulins having a desired affinity or specificity.

25. A method for producing libraries of human monoclonal antibody-producing lymphoma cells, said method comprising the step of expressing in a reverted human lymphoma cell a polynucleotide encoding a recombination-facilitating protein for a time and under conditions sufficient to induce rearrangement of genes encoding human antibodies in the reverted lymphoma cells.

26. A method for generating a library of lymphoma cell lines of human origin, each human lymphoma cell line capable of producing fully-human immunoglobulins of a particular specificity, said method comprising the steps of:

(a) reverting a human lymphoma cell line comprising a V(D)J rearranged immunoglobulin region by introducing a polynucleotide encoding human V, D, and/or J regions of human immunoglobulin heavy and/or light chains into said human lymphoma cell line, wherein said V, D, and/or J regions replace said V(D)J rearranged immunoglobulin region such that the introduced V, D, and/or J regions are in a pro-B cell-like or germline-like state; and

(b) expressing in the reverted human lymphoma cell a polynucleotide sequence encoding a human recombination-facilitating protein, or a functional fragment, derivative or variant thereof, for a time and under conditions sufficient to facilitate rearrangement of immunoglobulin-encoding genes thus generating a library of human lymphoma cells each producing a fully-human immunoglobulin of a particular specificity.

27. A method for generating a library of lymphoma cell lines of human origin, each human lymphoma cell line capable of producing fully-human immunoglobulin heavy chains of a particular specificity, said method comprising the steps of:

(a) reverting a human lymphoma cell line comprising a V(D)J rearranged immunoglobulin region in by introducing a polynucleotide encoding fused DJ regions of a human immunoglobulin heavy chain into said cell line, wherein said DJ regions replace said V(D)J rearranged immunoglobulin region such that the introduced fused DJ regions are in a pro-B cell-like state;

(b) expressing in the reverted human cell a polynucleotide sequence encoding a human recombination-facilitating protein, or functional fragment, derivative or variant thereof, for a time and under conditions sufficient to induce rearrangement of the human lymphoma cell line germline V regions with the introduced fused DJ regions, wherein rearrangement of the V and fused DJ regions facilitates expression of a fully-human immunoglobulin heavy chain.

28. A method for generating a library of lymphoma cell lines of human origin, each human lymphoma cell line capable of producing fully-human immunoglobulin light chains of a particular specificity, said method comprising the steps of:

(a) reverting a human lymphoma cell comprising a V(D)J rearranged immunoglobulin region in by introducing a polynucleotide encoding J regions of a human immunoglobulin light chain into said human lymphoma cell, wherein said J regions replace said V(D)J rearranged immunoglobulin region such that the introduced J regions are in a pro-B cell-like or a germline-like state;

(b) expressing in the reverted human lymphoma cell line a polynucleotide sequence encoding a human recombination-facilitating protein, or functional fragment, derivative or variant thereof, for a time and under conditions sufficient to induce rearrangement of the germline V regions with the introduced J regions, wherein rearrangement of the V and J regions facilitates expression of a fully-human immunoglobulin light chain.

29. The method of any one of claims 14-23 and 26-28 wherein reversion of cells to a pro-B cell-like state is achieved by introducing a vector comprising a polynucleotide encoding fused DJ regions of an immunoglobulin heavy chain, or fragment thereof.

30. The method of any one of claims 14, 16-20, 26, and 28 wherein reversion of cells to a germline-like state is achieved by introducing a vector comprising a polynucleotide encoding V, D, and/or J regions of immunoglobulin heavy and/or light chains, or fragments thereof, wherein the V, D, and/or J regions are assembled in the vector in a germline-like state.

31. The method of any one of claims 14, 16-20, 26, and 28 wherein reversion of cells to a germline-like state is achieved by introducing a chromosome, or substantial portion thereof, wherein said chromosome encodes a complete germline heavy or light chain antibody repertoire or substantial portion thereof.

32. The method of claim 31 wherein said chromosome is a human chromosome selected from the group consisting of chromosomes 2, 14, and 22.

33. The method of claim 31 wherein said chromosome replaces the corresponding endogenous V(D)J rearranged chromosome.

34. The method of claim 31 wherein said chromosome is introduced into said V(D)J rearranged cell by a methodology selected from the group consisting of microcell-mediated chromosome transfer, T cell-fusion, micro-injection, and yeast protoplast fusion.

35. The method of claim 31 wherein said cells comprising V(D)J rearranged immunoglobulin regions are reverted to a germline-like configuration by fusing a B cell line with precursor B cells isolated from human bone marrow or cord blood.

36. The method of claim 31 wherein said cell is reverted to a germline-like configuration by fusing a B cell line with T cells isolated from human blood.

37. The method of claim 31 wherein said cell is are reverted to a germline-like configuration by fusing B cell lines with rodent/human somatic cell hybrids carrying a single human chromosome.

38. The method of any one of claims 14-16 and 24 wherein the immunoglobulin heavy and/or light chain regions are human immunoglobulin heavy and/or light chain regions.

39. The method of any one of claims 14-16 and 24 wherein said immunoglobulin is from an animal selected from the group consisting of primate, sheep, pig, cow, horse, donkey, poultry, rabbit, mouse, rat, guinea pig, hamster, dog, and cat.

40. The method of any one of claims 14-18, 24-28 wherein said recombination-facilitating protein, or functional fragment, derivative, or variant thereof, is selected from the group consisting of RAG-1 and RAG-2.

41. The method of any one of claims 14-18, 24-28 wherein said polynucleotide encoding said recombination-facilitating protein, or functional fragment, derivative, or variant thereof, is introduced into said cell on an adenoviral vector.

42. The method of claim 41 wherein said recombination-facilitating protein, or functional fragment, derivative, or variant thereof, is from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, avian animals, reptilian animals, amphibian animals, and aquatic animals.

43. The method of claim 41 wherein said recombination-facilitating protein is human RAG-1 as depicted herein in SEQ ID NO: 2 or a functional fragment, derivative, or variant thereof.

44. The method of claim 41 wherein said recombination-facilitating protein is human RAG-2 as depicted herein in SEQ ID NO: 4 or a functional fragment, derivative, or variant thereof.

45. The method of claim 43 or 44 wherein said recombination-facilitating protein, or functional fragment, derivative or variant thereof, is expressed transiently for a time and under conditions sufficient to achieve recombination in said reverted cell.

46. The method of claim 43 or 44 wherein said recombination-facilitating protein, or functional fragment, derivative or variant thereof, is expressed constitutively and wherein expression is under the control of an inducible transcriptional promoter.

47. The method of any one of claims 15-29 wherein said immunoglobulin-producing cells are selected from the group consisting of lymphocytes, lymphocyte cell lines, B lymphocytes, B lymphocyte cell lines, B cell lymphoma cells, and B cell lymphoma cell lines.

48. The method of any one of claims 15-29 wherein said immunoglobulin-producing cells are a human B cell line.

49. The method of any one of claims 15-29 wherein said cells are generated from patients with Burkitt's lymphoma.

50. The method of any one of claims 15-29 wherein said cells are selected from the group consisting of Ramos, Ramos sub-line 2G6, Burkitt's lymphoma cell line BL2, Burkitt's lymphoma cell line BL16, and BL16 sub-line CL-01.

51. An immunoglobulin heavy and/or light chain generated by any one of the methods of any one of claims 15-17 and 25.

52. An immunoglobulin fragment generated from the immunoglobulin heavy and/or light chain of claim 51 wherein said immunoglobulin fragment is selected from the group consisting of an Fab, an F(ab')2, an Fc, and scFv fragment.

53. The method of any one of claims 18, 20-24, and 26-29 further comprising the step of treating said cells expressing said immunoglobulin heavy and/or light chains, or fragments thereof, with an agonist to induce switching from a first antibody isotype to a second antibody isotype wherein said first antibody isotype is selected from the group consisting of IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgE, IgA1 and IgA2 and wherein said

second antibody isotype is selected from the group consisting of IgD, IgG1, IgG2, IgG3, IgG4, IgE, IgA1 and IgA2.

54. The method of claim 53 wherein said agonist is selected from the group consisting of a ligand for CD40, a ligand for the B cell receptor, a B cell mitogen, a cytokines, bacterial DNA, a synthetic oligonucleotide containing unmethylated CpG dinucleotides, anti-CD19, and anti-CD21.

55. The method of any one of claims 18, 20-24, and 25-29 further comprising the step of subjecting to mutagenesis said cells or libraries of cells expressing one or more immunoglobulins, or fragments thereof.

56. The method of claim 55 wherein said mutagenesis step comprises introducing into the cell and/or library of cells a polynucleotide encoding activation-induced cytidine deaminase (AICD) or a functional fragment, derivative or variant thereof.

57. The method of claim 55 wherein said mutagenesis step is achieved by endogenous expression of an activation-induced cytidine deaminase (AICD).

58. The method of claim 57 wherein said endogenous expression of said activation-induced cytidine deaminase (AICD) is constitutive.

59. The method of claim 57 wherein said endogenous expression of said activation-induced cytidine deaminase (AICD) is induced.

60. The method of claim 58 further comprising the step of introducing into the cells and/or libraries of cells a polynucleotide encoding Terminal Deoxynucleotidyltransferase (TdT) or a functional fragment, derivative or variant thereof.